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# Vaccination for Leukemia

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## ABSTRACT

**Myeloid forms of leukemia would seem to be an ideal disease for investigators wishing to develop targeted immunotherapy because the leukemia is derived from antigen-presenting cells and because clinical data have demonstrated that there is potent T-cell immunity to chronic myeloid leukemia when donor lymphocyte infusions are used to treat relapse after transplantation. However, clinical vaccine studies have had to wait for the identification of specific antigens, some of which have recently been identified, and for a more complete understanding of basic tumor immunology. Here we review relevant fundamental T-cell biology, the nature of some important leukemia-associated antigens, and the preliminary results from recent clinical vaccine trials for leukemia. Although these studies are still early, they offer evidence that effective immunity to leukemia is possible after vaccination, thus setting the stage for future combined therapies.**


## KEY WORDS

Myeloid leukemia • Vaccination • Targeted immunotherapy • T lymphocytes

## INTRODUCTION

It has long been recognized that allogeneic stem cell transplantation conveys a potent graft-versus-leukemia (GVL) effect and a graft-versus-tumor (GVT) effect against some solid tumors. Nonmyeloablative stem cell transplantation strategies, in particular, rely on donor-mediated GVL and GVT to induce and maintain remission because the lower doses of chemotherapy that are used are not as effective as the more traditional high-dose myeloablative chemotherapy at controlling the disease. The use of donor lymphocytes to induce remission after relapse of chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) and the high relapse rate seen after T cell–depleted transplantation suggest that donor T lymphocytes are the principal mediators of GVL/GVT, although donor natural killer cells also contribute to GVL after haploidentical transplantation. Graft-versus-host disease (GVHD), also mediated by donor T cells, remains the major obstacle that must be overcome before we can exploit the full potential of GVL. Nevertheless, if we understood the nature of the target antigens of GVL, we could design rational vaccine strategies that could increase GVL. Our challenge is to understand the mechanism of GVL and GVHD, to determine whether these phenomena are separable, and to understand whether the T cells that mediate each of them can be identified and manipulated to produce a more favorable outcome after transplantation.

## BACKGROUND

To learn whether we can separate GVL from GVHD, we must first consider relevant clinical observations that give us clues as to the nature of GVL. The most compelling evidence of T cell–mediated GVL comes from studies of donor lymphocyte infusion (DLI) to treat relapsed CML. Lymphocyte transfusion from the original bone marrow donor induces both hematologic and cytogenetic responses in approximately 70% to 80% of patients with CML in chronic phase [1]. AML is also susceptible to the GVL effect, with 15% to 40% of patients obtaining remission with DLI alone [2]. In the case of CML, a complete cytogenetic response is usually obtained between 1 and 4 months after DLI [3], and approximately 80% of responders will achieve reverse transcriptase-polymerase chain reaction (PCR) negativity for the bcr-abl translocation [the fusion product of the

t(9;22) translocation found in CML] within a mean of 6 months [3]. In contrast, acute GVHD develops more rapidly, often within days or weeks after successful engraftment. It is interesting to note that, like complete remission, chronic GVHD develops over several months and is most closely correlated with an increased disease-free survival. Moreover, chronic GVHD often resembles autoimmune diseases such as scleroderma and arthritis. Whereas significant GVHD occurs in 50% of most patients treated with DLI and disease response occurs in 90% of CML patients, 55% of patients who do not experience GVHD also have a disease response [4,5]. From these observations, we can conclude that GVL develops slowly, generally over 2 to 6 months, and like most other therapies it works best when the patient has minimal residual disease. This might be the result of a low precursor frequency of GVL-producing T cells in the donor repertoire, which require enough time to proliferate to sufficient numbers in the recipient. Data from clinical trials that used large numbers of tumor-infiltrating lymphocytes to successfully treat melanoma patients support this conclusion. Furthermore, the clinical data summarized above show that GVL is separable from GVHD in some patients. Together, these observations suggest the potential to manipulate donor T-cell immunity in favor of GVL.

Animal models have established that both donor $CD4^+$ and $CD8^+$ αβ T-cell antigen receptor (TCR)–positive T cells can mediate GVL, and clinical studies of T cell–depleted stem cell transplants have confirmed the central role of αβ T cells.

These $CD4^+$ and $CD8^+$ αβ T cells recognize peptide antigens in the context of class II and class I major histocompatibility complexes (MHC), respectively. Peptides derived from cytoplasmic proteins that are 8 to 11 amino acids long bind in the groove of class I MHC molecules and are transported via the endoplasmic reticulum to the cell surface. Larger peptides, typically 12 to 18 amino acids long, that are derived from the processing of extracellular proteins bind class II MHC molecules and are presented to T cells on the cell surface. Both peptide/MHC I and peptide/MHC II are recognized by the heterodimeric αβ TCR on CD8 or CD4 T lymphocytes, respectively, with weak affinity and rapid off rates. Points of contact between the TCR and the peptide/MHC surface include surface amino acids contributed by the 2 α-helical domains of the MHC molecule that flank the peptide antigen-binding pocket, as well as amino acids from the peptide itself. In the case of MHC-matched allogeneic transplantation, single–amino acid differences in similar peptides, most often the result of nonsynonymous polymorphic differences in DNA between donor and recipient, account for much of the alloreactivity.

Our understanding of the nature of antigen-specific T-cell responses has been greatly improved by the discovery that antigen-specific TCRs can be reversibly labeled with soluble peptide/MHC tetramers [6]. Peptide antigen, $\beta_2$-microglobulin, and the MHC I heavy chain are folded together and, via a biotinylation signal sequence at the C-terminus of the MHC I heavy chain, are linked covalently to streptavidin in a 4:1 molecular ratio. When the streptavidin molecule is linked to a fluorescent dye such as phycoerythrin, the resulting peptide/MHC tetramers can be used to identify antigen-specific T cells by fluorescence-activated cell-sorting analysis because of their higher binding avidity to the cognate TCR. By using tetramers, it has been determined that up to 45% of all peripheral circulating T cells may be specific for a single dominant antigen at the height of an immune response to Epstein-Barr virus infection [7], and similar dominance may be seen during other viral infections [8,9]. Tetramers have also been used to study immune responses to tumor antigens [10], and they have also aided in their discovery [11].

## CANDIDATE VACCINE ANTIGENS

Various candidate antigens have been identified in leukemia immunity. For instance, tissue-restricted minor histocompatibility antigens (mHAs) that are derived from proteins expressed only in hematopoietic tissue have been shown to be the targets of alloreactive T cells [12-16]. These mHAs often result from polymorphic differences between donor and recipient in the coding regions of peptide antigens that bind within the groove of MHC molecules and are recognized by donor T cells. Recently, however, a newly described mHA was found to result from differential expression in donor and recipient as a result of a gene deletion [12]. Heterologous T-cell clones that demonstrate alloreactivity toward mHAs have been established from patients with severe GVHD after bone marrow transplantation with an HLA-matched donor [17-20]. Some of these mHA-specific cytotoxic T lymphocyte (CTL) clones react only with hematopoietic-derived cells, thus suggesting tissue specificity [19] and, therefore, potentially shared antigens on leukemia. In one study, GVHD correlated closely with differences in the minor antigen HA-1 in HLA-identical sibling transplantations [21]. Expression of 2 human mHAs, identified as HA-1 and HA-2, is confined to hematopoietic tissues, and HA-2 was identified as a peptide derived from the non–filament-forming class I myosin family by using mHA-reactive CTL clones to screen peptide fractions eluted from MHC class I molecules [14]. It is yet unclear whether CTLs specific for the minor antigens identified thus far convey only leukemia-specific immunity without concomitant GVHD. Immunization of leukemia patients after allogeneic stem cell transplantation (termed *vaccination by proxy* by David Scheinberg of Memorial

Sloan Kettering Cancer Center, 2003) with mHAs might promote GVL and reduce GVHD if appropriate hematopoietic-restricted mHA could be targeted (such as HA-1 or HA-2). In a recent report of 3 CML patients who received DLI after relapse, however, GVHD occurred in each patient, albeit grade II or less [22], concomitant with an increase in HA-1–specific or HA-2–specific CTLs and cytogenetic remission. A practical limit of vaccination immunotherapy with these mHAs is that only 10% of individuals would be expected to have the relevant HA-1 alternate allele, and <1% would have the HA-2 alternate allele, which makes potential donor availability quite limiting.

Several investigators have used BCR-ABL peptides to elicit CML-specific T-cell responses. Because BCR-ABL is present in nearly all Philadelphia chromosome–positive CML patients, it is thought to represent a potentially unique leukemia antigen. The ABL coding sequences upstream (5′) of exon II on chromosome 9 are translocated to chromosome 22 and fused in-frame with the BCR gene downstream (3′) of exon III, resulting in the most common chimeric messenger RNA transcript (b3a2), which is translated into a chimeric protein ($p210^{BCR\text{-}ABL}$). Translation of b3a2 messenger RNA results in the coding of a unique amino acid (lysine) within the fusion region. Some HLA-A2–, -A3–, -A22–, and -B8–restricted overlapping peptides inclusive of this lysine could bind to their respective HLA alleles and could be used to elicit T-cell proliferative responses when the peptide was either pulsed onto HLA-matched normal antigen-presenting cells or onto HLA-B8–positive CML cells [23-25]. However, when the b3a2 peptides were used to elicit b3a2-specific T lymphocyte lines in vitro, the resulting T cells could not specifically lyse fresh CML cells that had not previously been pulsed with the peptide [25]. This could be due to a low affinity of the peptide-specific CTL, or the peptide may not be processed or presented on CML cells. More recently, however, b3a2-specific CTLs were identified in the peripheral blood of chronic phase CML patients by using soluble b3a2 peptide/MHC tetramers [26]. Although the tetramer-positive CTLs from the patients were not examined for their ability to kill autologous CML target cells, b3a2-specific CTL elicited in vitro from healthy donors were able to kill CML cells. This suggests that bcr-abl fusion peptides may also be targets of CTL immunity. Further evidence by Gannage et al. [27] shows that bcr-abl–specific CTLs can be identified in the peripheral blood of 61% of CML patients.

To adapt what has been learned about immunity against solid tumor antigens to the study of myeloid leukemia antigens, we studied myeloid-restricted proteins that are aberrantly expressed in leukemia relative to normal hematopoietic progenitors. Myeloid leukemia, like normal myeloid progenitors, expresses a number of differentiation antigens associated with granule formation. Two examples of aberrantly expressed antigens in human leukemia are proteinase 3 (PRTN3) and neutrophil elastase (ELA2), neutral serine proteases that are stored in primary azurophil granules and maximally expressed at the promyelocyte stage of normal myeloid differentiation [28-30]. PRTN3 and ELA2 are coordinately regulated, and the transcription factors PU.1 and C/EBPα, which are responsible for normal myeloid differentiation from stem cells to monocytes or granulocytes, are important in mediating their expression [31]. These transcription factors have been implicated in leukemogenesis [32], and PRTN3 may be important in maintaining a leukemia phenotype because PRTN3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukemia cell line [33]. Similarly, ELA2 is overexpressed in the serum of leukemia patients, and it preferentially suppresses normal hematopoietic progenitors and is involved in modulating CXCR4-mediated homing of stem cells to bone marrow.

What may be critical for our ability to identify T-cell antigens in these proteins is the observation that PRTN3 and ELA2 are targets of autoimmune diseases such as Wegener granulomatosis [34] and small-vessel vasculitis [35-37]. There is evidence for both T-cell and humoral immunity in patients with these diseases. Wegener granulomatosis is associated with production of cytoplasmic antineutrophil cytoplasmic antibodies with specificity for PRTN3 [38]. T cells taken from affected individuals proliferate in response to crude extracts from neutrophil granules and to purified proteins [36,39]. These findings suggest that T-cell responses against these proteins might be relatively easy to elicit in vitro by using a deductive strategy to identify HLA-restricted peptide epitopes. On the basis of this hypothesis, we identified PR1, an HLA-A2.1–restricted nonamer derived from both PRTN3 and ELA2, as a leukemia-associated antigen [11,40-42] by first searching the length of the protein with use of the HLA-A2.1 binding motif, the most prevalent HLA allele. Peptides predicted to have high-affinity binding to HLA-A2.1 were synthesized, confirmed to bind, and then used to elicit peptide-specific CTLs in vitro from healthy donor lymphocytes. We have found that PR1 can be used to elicit CTLs from HLA-$A2.1^+$ normal donors in vitro and that T-cell immunity to PR1 is present in healthy donors and in many patients with CML who are in remission. These PR1-specific CTLs show preferential cytotoxicity toward allogeneic HLA-$A2.1^+$ myeloid leukemia cells over HLA-identical normal donor marrow [40]. In addition, PR1-specific CTLs inhibit colony-forming unit granulocyte-macrophage from the marrow of CML patients, but not from normal

HLA-matched donors [41]; this suggests that leukemia progenitors are also targeted.

Using PR1/HLA-A2 tetramers to detect CTLs specific for PR1 (PR1-CTLs), we found a significant correlation between cytogenetic remission after treatment with interferon α and the presence of PR1-CTLs [11]. Somewhat surprisingly, PR1-CTLs were also identified in the peripheral blood of some allogeneic transplant recipients who achieved molecular remission and who had converted to 100% donor chimerism. PR1/HLA-A2 tetramer-sorted allogeneic CTLs from patients in remission were able to kill CML cells but not normal bone marrow cells in 4-hour cytotoxicity assays, thus demonstrating that the PR1 self-antigen is also recognized by allogeneic CTLs [11]. These studies established PR1 as a human leukemia–associated antigen and established that PR1-specific CTLs contribute to the elimination of CML [11].

Several other HLA-restricted epitopes have been identified as potentially relevant leukemia-associated antigens. The Wilms tumor antigen 1 (WT-1) has emerged as a very potent immunogen containing multiple unique HLA-restricted epitopes [43-47], and it may also be a marker of minimal residual disease because it is aberrantly expressed in both myeloid and lymphoid acute leukemia [48-50]. These features make it an attractive vaccine candidate. In addition, the telomerase antigen human Telomerase Reverse Transcription (hTERT) is aberrantly expressed in leukemia and nearly all solid tumors, and hTERT-specific $CD8^+$ T cells kill leukemia target cells. Although hematopoietic stem cells and T cells normally express high levels of hTERT, they are not killed in vitro. Various surface molecules on leukemia cells, such as CD45, present on all hematopoietic cells, and CD33 and CD19 on myeloid and lymphoid cells, respectively, have also been studied by deductive means to uncover potentially immunogenic epitopes [51-53]. Although some HLA-restricted epitopes have been identified, it is not yet clear whether any of these are leukemia-associated antigens. The method of serologic screening of complementary DNA expression libraries with autologous serum (SEREX) has also been used to identify melanoma-associated antigen (MAGE-1) and to confirm WT-1 as a potential leukemia-associated antigen, although there may be some controversy over whether the MAGE proteins are expressed in leukemia blasts [54].

## CLINICAL TRIALS

The PR1 peptide is undergoing phase I/II study, and the single-peptide epitope is combined with incomplete Freund adjuvant and granulocyte-macrophage colony-stimulating factor and administered every 3 weeks for a total of 3 total vaccinations. Patients with AML, CML, and myelodysplastic syndrome are eligible, and the first 9 patients were reported at the annual American Society of Hematology meeting in 2002; patient follow-up was updated in 2004 [55]. To judge whether a clinical response was due to the vaccine, eligible patients were required to have progression, relapse, or second complete remission or beyond (AML patients only) before vaccination. Immune responses, measured with PR1/HLA-A2 tetramers, were noted in 5 patients, and clinical remissions were noted in 4. Notably, the TCR avidity of the vaccine-induced PR1-specific CTLs was higher in the clinical responders than in the nonresponders, and durable molecular remissions were noted in 2 patients with refractory AML who were followed up for 8 months to nearly 3 years. Although the trial is still ongoing, both central and effector memory PR1-CTLs seem critical for successful vaccination and lasting immunity.

Results of early clinical trials with WT-1–derived peptides and with BCR-ABL fusion peptides also show very interesting results. In a heterogeneous group of 26 patients with AML and myelodysplastic syndrome, immune responses were quantified by tetramer staining, and reductions in blasts were observed after 2 to 3 peptide vaccinations of WT-1 peptides plus adjuvant given every 2 weeks [56]. Although many patients in this trial were treated in hematologic remission, a reduction in the amount of WT-1 transcript, measured by PCR, correlated with immune response. Immunity to BCR-ABL peptides has also been elicited in clinical trials. By immunizing CML patients with 6 different b3a2 fusion peptides plus adjuvant, immune responses were noted in all 14 patients, although clinical efficacy could not be clearly established [57]. In another, more recent, trial, 17 patients with CML were given 6 vaccinations of b3a2 fusion region peptides plus adjuvant, and immune responses were measured by enzyme-linked ImmunoSPOT (ELISPOT) or tetramer. The patients had stable residual disease that could be measured by standard cytogenetics or quantitative PCR after treatment with imatinib or interferon-α. Complete cytogenetic remission was observed in 7 patients after vaccination, although the patients continued to receive imatinib or interferon α. Nevertheless, peptide-specific immune responses were noted in these patients, and they were associated with clinical responses [58].

## CONCLUSIONS

In summary, an increased understanding of the critical elements in T-cell immunity against leukemia has led to the identification of a handful of candidate leukemia antigens. In preliminary clinical trials, PR1, WT-1, and BCR-ABL seem to be promising peptide vaccine antigens with little or no toxicity. It is impor-

tant to note that patients who experience clinical benefit are those with minimal disease who may have an intact T-cell repertoire with the potential to recognize the peptide antigen/MHC. This observation may in part explain why peptide vaccination of leukemia may hold greater potential than similar vaccine strategies for solid tumors, where objective responses are observed in <3% of patients overall, because sensitive PCR-based measurements that establish minimal residual disease in leukemia patients provide a window of opportunity for vaccination. Furthermore, because the malignant cells and the potential immune responder cells reside within the same primary and secondary lymphatic organs, T cells do not need to traffic to distant tumor sites to find and kill the leukemia cells. It will be important to confirm whether vaccines hold potential as part of the therapy of leukemia by conducting randomized trials with proper immune monitoring that can establish vaccine-induced immunity. In the future, combination therapy with targeted small molecules, viewed mostly as drugs that target critical molecular pathways in malignant cells, may also include vaccine antigens.

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